View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

D021

SILDENAFIL INDUCED-REVASCULARIZATION OF RAT HINDLIMB INVOLVED ARTERIOGENESIS THROUGH PI3K/-AKT AND ENOS ACTIVATION

C. MENGUY [1], A. BOCQUET [1], A.-L. GUIHOT [1], B. TOUTAIN [1], M. ROLLI-DERKINDEREN [2], D. CHAPPARD [3], P. PACAUD [2], G. LOIRAND [2], D. HENRION [1], L. LOUFRANI [1]
[1] *UMR CNRS 6214 Inserm 771, Angers, France*
[2] *Inserm U915, Nantes, France*
[3] *Inserm EMI 0335, Angers, France*

Hypoxia and inflammation play a major role in the revascularization following ischemia. Sildenafil inhibits phosphodiesterase-5, increases intracellular cGMP content and thus induces vasodilation. Sildenafil also induces neovascularization following ischemia but through a pathway remaining incompletely understood. Thus, we investigated the consequences of a long-term sildenafil treatment on post-ischemic revascularization.

The left femoral artery was ligated in sildenafil (25 mg/kg per day)-treated rats. Vascular density and blood flow were evaluated in both legs and expressed as left/right leg (L/R) ratio. After 7 or 21 days, the L/R ratio was 33¡Ó2% and 54¡Ó9%, respectively in control rats. Sildenafil increased significantly the ratio to 47¡Ó4% and 128¡Ó11%, respectively. A neutralizing VEGF antibody significantly decreased vascular density (x0.48-fold) in control rats without affecting density in sildenafil-treated animals. Blood flow and arteriolar density followed the same pattern. In the ischemic leg, HIF1*f*Ñ and VEGF expression level increased in control, not in sidenafil ¡Vtreated rats, suggesting that sildenafil might not preferentially induce angiogenesis. PI3-kinase, Akt and eNOS were activated after 7 days with a down-regulation after 21 days. Sildenafil-induced migration of endothelial cells was prevented by PI3-kinase inhibition with LY294002. Finally, sildenafil-induced rise in blood flow in mesenteric resistance arteries was associated with an increased luminal diameter (outward remodeling or arteriogenesis). This arteriogenesis was also associated with eNOS proteins activation.

Conclusion — Long term sildenafil treatment increased local blood flow and collateral arteries growth independent of VEGF but in association with activation of PI3-kinase, Akt and eNOS which might preferentially activate arteriogenesis.

D022

NATURAL CD4/CD25/FOXP3 REGULATORY T CELLS MODULATE POST-ISCHEMIC INFLAMMATORY RESPONSE: ROLE IN NEOVASCULARIZATION

Y. ZOUGGARI [1], H. AIT-OUFELLA [1], L. WAECKEL [1], J. VILAR [1], C. LOINARD [1], C. COCHAIN [1], A. RECALDE [1], M. DURIEZ [1], B. LEVY [1], E. LUTGENS [2], Z. MALLAT [1], J.-S. SILVESTRE [1]
[1] *Cardiovascular Research Center Inserm U689 Lariboisiere, Paris, France*
[2] *Department of Pathology, Cardiovascular Research Institute Maastricht, Maastricht, The Netherlands*

CD4+ and CD8+ T lymphocytes control revascularization after vascular occlusion. T cell activation is mediated by two major costimulatory signalings: the B7/CD28 and the CD40-CD40 ligand pathways. Interestingly, CD28 interactions with the structurally related ligands B7-1 and B7-2 are also required for the generation and homeostasis of CD4+CD25+ regulatory T cells (Treg), which actively maintain immunological tolerance to self and nonself antigens. We hypothesized that naturally arising Treg modulate the immuno-inflammatory response to ischemic injury, and subsequently vessel growth.

Ischemia was induced by right femoral artery ligation in CD28-deficient mice (n=10 per group). After 21 days of ischemia, CD28 deficiency showed a profound reduction in Treg number and upregulated post-ischemic inflammatory response and neovascularization. Similarly, injection of splenocytes isolated from CD28-/- mice in Rag1-/- mice with hindlimb ischemia increased angiographic score, foot perfusion, and capillary density by 2.2-, 2.3- and 1.1-fold, respectively, compared to PBS-injected Rag1-/- mice. These effects were associated with enhanced accumulation of CD3-positive T cells and Mac-3 positive macrophages in the ischemic leg of Rag1-/- mice treated with CD28-/- splenocytes. Interestingly, cotransfer of Treg with CD28-/- splenocytes in Rag1-/- mice abrogated activation of neovascularization induced by CD28-/- splenocytes. Inflammatory cells accumulation was also decreased in Rag1-/- transplanted with both Treg and CD28-/- splenocytes compared to mice receiving CD28-/- splenocytes only. In contrast, treatment of C57Bl/6 Wild-Type mice with an anti-CD25 antibody (PC61) markedly reduced endogenous Treg levels in blood and spleen. At day 14 of ischemia, inflammatory response and neovascularization were markedly increased in anti-CD25 treated Wild-Type mice compared to untreated mice. These results provide new insights into the immunoregulation of post-ischemic neovascularization.

D023

CHOP-10 DELETION IMPROVES NEOVASCULARIZATION AND STEM/PROGENITOR CELLS PRO-ANGIOGENIC POTENTIAL IN TYPE I DIABETIC MICE WITH HINDLIMB ISCHEMIA

C. LOINARD [1], C. HEYMES [1], J. VILAR [1], T. EBRAHIMIAN [2], P. RUEDA [3], Y. ZOUGGARI [1], C. COCHAIN [1], M. DURIEZ [1], B. LEVY [1], F. ARENZANA-SEISDEDOS [3], J.-S. SILVESTRE [1]
[1] *Centre de Recherche Cardiovasculaire Inserm U689, Paris, France*
[2] *Laboratoire de radiopathologie, institut de radioprotection et de sûreté nucléaire (IRSN), Fontenay-aux-Roses, France*
[3] *Institut Pasteur Unité de Pathogénie Virale Moléculaire, Paris, France*

Diabetes-induced reactive oxygen species overproduction impairs neovascularization. CHOP 10 is a novel developmentally regulated nuclear protein that emerges as critical transcriptional integrator among pathways regulating differentiation, proliferation and survival. Of interest, CHOP-10 has been shown to trigger oxidative stress-induced β cells apoptosis in the setting of diabetes. Here, we analyzed the role of CHOP-10 in postnatal neovascularization and bone-marrow-derived mononuclear cells (BMC) pro-angiogenic potential in type I diabetic mice with hindlimb ischemia.

Ischemia was induced by right femoral artery ligation in C57/Bl6 animals (WT, n=8), diabetic C57/Bl6 animals (diab WT, n=8, Streptozotocin 40 mg/kg) and diabetic CHOP-10—deficient animals (diab CHOP-10 KO, n=8). Two days after ischemia, CHOP-10 mRNA and protein levels were increased by 7- ($p<0.001$) and 4-fold ($p<0.01$), respectively in ischemic muscle of WT diab compared to WT. Angiographic score, capillary density and foot perfusion were increased by 3.3- ($p<0.01$), 1.8- ($p<0.001$) and 2.2-fold ($p<0.001$)

in diab CHOP-10 KO compared to WT diab, 21 days after ischemia. This effect was associated with a reduction in the number of apoptotic cells and an increase in eNOS levels in diab CHOP-10 KO compared to WT diab. We next analyzed the role of CHOP-10 in post-natal vasculogenesis. Injection of BMC isolated from diab CHOP-10 KO in WT mice with hindlimb ischemia improved neovascularization by around 1.8-fold when compared to WT diab BMC ($p<0.05$). BMC isolated from diab CHOP-10 KO mice showed an upregulation of eNOS protein levels and an increase in their ability to differentiate into cells with endothelial phenotype in vitro differentiation assay. Finally, treatment of cultured HUVEC with homocysteine increased CHOP-10 mRNA levels and repressed eNOS gene expression. Consistent with these results, eNOS protein expression was significantly upregulated in the CHOP-10 siRNA—transfected endothelial cells. Additionally, overexpression of CHOP-10 inhibited the basal transcriptional activation of the eNOS promoter as assessed by a reporter gene assay using a 3,500-bp fragment of the human eNOS gene.

Our study unravels an important inhibitory role of CHOP-10 in the regulation of vessel formation in the setting of diabetes.

D024

LYSYL OXIDASE LIKE 2 REGULATES VASCULAR CELLS MIGRATION AND BASAL LAMINA ORGANISATION

M. BIGNON [1], J. HARDOUIN [2], N. BRECHOT [1], L. NASCIUTTI [3], R. JOUBERT-CARON [2], M. CARON [2], S. GERMAIN [1, 4], C. MONNOT [1], L. MULLER [1]

[1] *Inserm U833 — Collège de France, PARIS, FRANCE,* [2] *LBPP — UMR CNRS 7033 Université Paris XIII, Bobigny, France*

[3] *Universidade Federal do Rio de Janeiro, Rio De Janeiro, Brazil*

[4] *Service d'Hématologie Biologique A — Hôpital Européen Georges Pompidou — AP-HP, Paris, France*

Hypoxia stimulates angiogenesis during development, as well as in the course of ischemic cardiovascular diseases and tumor growth. In a hypoxic environment, endothelial cells (EC) are key players of the angiogenic response. Their activation leads to remodeling of the extracellular matrix (ECM): a degradation step generates a provisional ECM supporting EC proliferation and migration; assembly of a new basal lamina leads to pericyte recruitment and neovessel maturation.

In order to identify endothelial ECM proteins regulated by hypoxia, 2D gel electrophoresis was performed on ECM samples prepared from cultured EC of micro or large vessels (HDMEC or HUVEC respectively). Mass spectrometry identified lysyl oxidase-like protein 2 (LOXL2) as a highly hypoxia-induced protein. Lysyl oxidases are secreted enzymes involved in ECM maturation through covalent cross-linking of its major components, collagens and elastin. The induction of LOXL2 expression was detected both at the mRNA and protein levels. Using siRNA, we demonstrated that LOXL2 is responsible for 65% of lysyl oxidase total activity in hypoxic EC. In addition, LOXL2 protein was colocalised with type IV collagen in endothelial ECM.

We further investigated the expression of LOXL2 in vivo. The enzyme was expressed in rat EC from retina during postnatal vascular development, and from adult skeletal muscle. In a murine model of hindlimb ischemia, LOXL2 was upregulated at the protein and mRNA levels. In situ hybridization revealed its expression in both EC and macrophages.

Involvement of LOXL2 at different steps of the angiogenic process was further studied in vitro. We demonstrated that LOXL2 increases EC migration on fibronectin, as well as migration and tube formation in fibrin 3D gels. In addition, LOXL2 knock-down inhibited type IV collagen deposition in the ECM, suggesting a major role for LOXL2 in the organisation of endothelial basal lamina. Finally, whereas the efficiency of endothelial ECM for recruiting VSMC was increased by hypoxia, this effect was reduced upon knocking down endothelial LOXL2 expression. Altogether, these data suggest that LOXL2 plays a major role in EC migration, vascular basal lamina deposition and neovessel stabilisation during hypoxia-induced angiogenesis.

D025

PHAGOCYTOSIS IS PIVOTAL IN THE BENEFICIAL EFFECT OF BONE MARROW MONONUCLEAR CELLS-BASED THERAPY FOR MYOCARDIAL INFARCTION

C. COCHAIN [1], P. BONIN [1], J. VILAR [1], A. RÉCALDE [1], Y. ZOUGGARI [1], C. LOINARD [1], A. RICHART [1], V. POURESMAIL [1], M. DURIEZ [1], B. LÉVY [1], Z. MALLAT [1], J.-S. SILVESTRE [1]

[1] *Inserm U689, Paris, France*

Cell-based therapy is a promising option for treatment of cardiovascular diseases. Based on experimental studies demonstrating that bone marrow-derived mononuclear cells (BMMNCs) improve the functional recovery after ischemia, clinical trials were initiated to address this new therapeutic concept. BMMNCs improve neovascularization of ischemic tissue by a broad repertoire of potential therapeutic actions. Whereas initial studies documented that the cells incorporate and differentiate to cardiovascular cells, other studies suggested that short-time paracrine mechanisms mediate the beneficial effects. Here, we hypothesized that BMMNCs have a phagocytic ability, and switch to a proangiogenic phenotype after engulfment of apoptotic cells. Activation of such angiogenic program may be pivotal in the beneficial effect of BMMNCs-based therapy. In vitro, wild-type (WT) BMMNCs ingestion of apoptotic cells upregulated the release of proangiogenic factors VEGF and HGF by 15- and 5-fold, respectively. In contrast, BMMNCs collected from mice deficient in MFG-E8, a protein that is required for attachment and engulfment of apoptotic cells by phagocytes, displayed lower phagocytic ability, leading to decrease in VEGF and HGF release. The capacity of BMMNCs to differentiate into cells with endothelial phenotype was similar in control and MFG-E8-deficient cells. In an in vivo model of mice myocardial infaction (MI), transplantation of WT BMMNCs increased fractionnal shortening (120% of untreated control, $p<0.05$), 14 days after MI. Size of the infarct scar was reduced by 30% ($p<0.05$ vs untreated control), and capillary density in the infarct border zone was raised by 10% ($p<0.05$ vs untreated control) in the WT BMMNCs group. In contrast, transplantation of MFG-E8 deficient BMMNCs displayed no significant effect on cardiac function, infarct size or angiogenesis in the ischemic myocardium. Four days after MI, VEGF protein levels were raised by 1.4 fold in the myocardium of WT BMMNCs treated animals compared to untreated controls ($p<0.05$), while treatment with MFG-E8 deficient BMMNCs failed to raise VEGF levels. Taken together, these results suggest that phagocytosis of apoptotic cells reprograms BMMNCs into a proangiogenic phenotype. Hence, the therapeutic effect of transplanted BMMNCs depends, at least in part, on their phagocytic ability.